METHODS FOR CHARACTERIZING LOW-ABUNDANCE HOST CELL PROTEINS

FIELD

[0001] The present invention generally pertains to methods and systems for identifying and quantitating low-abundance host cell proteins (HCP) to monitor and control impurities in biopharmaceutical products.

BACKGROUND

[0002] Recombinant DNA technology has been used widely for producing biopharmaceutical products in host cells. Biopharmaceutical products must meet very high standards of purity. Thus, it can be important to monitor any impurities in such biopharmaceutical products at different stages of drug development, production, storage and handling. Residual impurities should be at an acceptable low level prior to conducting clinical studies. For example, host cell proteins (HCPs) can be present in protein-based biopharmaceuticals which are developed using cell-based systems. The presence of HCPs in drug products need to be monitored and can be unacceptable above a certain amount. Sometimes, even trace amounts of HCPs can cause an immunogenic response.

[0003] Immuno-assays have been used to monitor HCP removal using polyclonal anti-HCP antibodies. Immuno-assays can provide semi-quantitation of total HCPs levels in high throughput, but they may not be effective in quantitating individual HCPs rapidly. Liquid chromatography-mass spectrometry (LC-MS) has recently emerged for monitoring HCP removal. However, the enormous dynamic concentration ranges of HCPs in the presence of high concentration of purified antibodies can be a challenge for developing LC-MS method to monitor the removal of HCPs.

[0004] It will be appreciated that a need exists for methods and systems to identify and quantitate HCPs to monitor and control the residual HCPs in drug substance to mitigate safety risks.

SUMMARY

[0005] The identification of HCP impurities in biopharmaceutical products encounters challenges of dealing with the broad dynamic range of protein concentrations due to very high sample complexity. The present application provides methods and systems to identify HCP impurities in a sample containing high-abundance proteins including an enrichment method to fulfill the need of enriching low abundance HCPs in therapeutic drug products.

[0006] This disclosure provides a method of identifying and/or quantifying HCP impurities in a sample. In one exemplary embodiment, the method of identifying and/or quantifying HCP impurities in a sample comprises: contacting the sample to solid support, wherein interacting peptide ligands have been attached to the solid support and the HCP impurities can bind to the interacting peptide ligands; washing the solid support using a solution comprising a surfactant to isolate HCP impurities and provide an eluent; subjecting the eluent to an enzymatic digestion reaction to generate components of the isolated HCP impurities; and identifying the components of the isolated HCP impurities using a mass spectrometer; wherein the sample comprises at least one high-abundance peptide or protein. In one aspect, the surfactant in the method of the present application is a phase transfer surfactant, an ionic surfactant, an anionic surfactant, a cationic surfactant or combinations thereof. In another aspect, the surfactant in the method of the present application is sodium deoxycholate, sodium lauryl sulfate or sodium dodecylbenzene sulphonate.

[0007] In one aspect, a concentration of the at least one high-abundance peptide or protein in the method of the present application is about at least 1000 times, about 10,000 times, about 100,000 times or about 1,000,000 times higher than a concentration of the each HCP impurity. In one aspect, the interacting peptide ligands in the method of the present application are a library of combinatorial hexapeptide ligands. In another aspect, the HCP impurities in the method of the present application are quantified using a mass spectrometer, wherein a detection limit of each HCP impurity is about 0.05-0.1 ppm. In another aspect, the at least one high-abundance peptide or protein in the method of the present application is an antibody, a bispecific antibody, an antibody fragment, a Fab region of an antibody, an antibody-drug conjugate, a fusion protein, a protein pharmaceutical product or a drug. In one aspect, an enzyme of the enzymatic digestion reaction in the method of the present application is trypsin.

[0008] In yet another aspect, the mass spectrometer in the method of the present application is an electrospray ionization mass spectrometer, nano-electrospray ionization mass spectrometer or a triple quadrupole mass spectrometer, wherein the mass spectrometer is coupled to a liquid chromatography system. In one aspect, the mass spectrometer is capable of performing LC-MS (liquid chromatography-mass spectrometry) or a LC-MRM-MS (liquid chromatography-multiple reaction monitoring-mass spectrometry) analyses.

[0009] This disclosure, at least in part, provides a system for identifying HCP impurities in a sample. In one exemplary embodiment, the system for identifying HCP impurities in a sample comprises: a solid support; interacting peptide ligands, wherein the interacting peptide ligands are attached to the solid support such that the HCP impurities can bind to the interacting peptide ligands; a solution comprising a surfactant capable of washing the solid support to isolate HCP impurities; an enzymatic digestion solution capable of generating components from the isolated HCP impurities; and a mass spectrometer capable of identifying or quantifying the components from the isolated HCP impurities; wherein the sample comprises at least one high-abundance peptide or protein.

[0010] In one aspect, the surfactant in the system of the present application is a phase transfer surfactant, an ionic surfactant, an anionic surfactant, a cationic surfactant, or combinations thereof. In one aspect, the surfactant in the system of the present application is sodium deoxycholate, sodium lauryl sulfate, or sodium dodecylbenzene sulphonate. In another aspect, a concentration of the at least one high-abundance peptide or protein in the system of the present application is about at least 1000 times, about 10,000 times, about 100,000 times or about 1,000,000 times higher than a concentration of the each HCP impurity. In another aspect, the interacting peptide ligands in the system of the present application are a library of combinatorial hexapeptide ligands. In one aspect, a detection limit of each HCP impurity in the system of the present application is about 0.05-0.1 ppm. In yet another aspect, the at least one high-abundance peptide or protein in the system of the present application is an antibody, a bispecific antibody, an antibody